

CXL. THE OXIDATION OF OLEIC ACID BY MEANS OF HYDROGEN PEROXIDE WITH AND WITHOUT THE ADDITION OF COPPER SULPHATE: A POSSIBLE ANALOGY WITH ITS OXIDATION *IN VIVO*.

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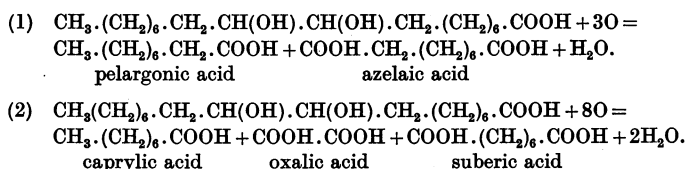
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THE discussion of the method of biological oxidation of the fatty acids has hitherto been almost entirely confined to the saturated acids, in spite of the fact that the acid which occurs in greater proportion than any other in the large majority of fats is the unsaturated oleic acid. Such facts as are available point to the conclusion that where oxidation is taking place rapidly the fat contains a relatively large proportion of unsaturated acid. Lewkowitsch quotes figures, for instance, showing that the fat of animals in the wild state is more unsaturated than that of the same species reared in domesticity: the iodine value of the fat of the wild rabbit is 101 as compared with 64 for the fat of a tame rabbit, and similar differences hold for the goose and the hog. Although it has been established [Ellis and Zeller, 1930] that carbohydrate feeding leads to a diminution of the unsaturated acids in the storage fat, it is uncertain whether variations in diet would account for the whole of the above differences, and it is possible that where the animal is leading a more active life a higher proportion of unsaturated acids is available. In the fat of the lower organisms the proportion of unsaturated acid is greater when they are grown at a low temperature and diminishes as the temperature at which they are grown is raised [Terroine, *et al.* 1927; Pearson and Raper, 1927]. Blood-plasma contains a high proportion of unsaturated acids, a large proportion of which are more unsaturated than oleic acid [Bloor, 1924]. In the liver oils the proportion of saturated acids is comparatively low. Bloor [1926, 1927] has shown that in the fat extracted from heart muscle and from voluntary muscle it is the unsaturated acids which predominate, the proportion being greater than in storage fat. The oxidation of the unsaturated acids in the body appears to be a normal process and it is generally held, largely owing to the work of Leathes and his colleagues, that desaturation of the fatty acids precedes oxidation. Nothing, however, is known of the stages by which oleic acid breaks down in the body and the theory of β -oxidation, which has found most acceptance as the probable method by which the saturated acids containing normal chains

are oxidised, does not necessarily hold good in the case of the unsaturated acids, since here oxidation must first take place at the ethylenic linkage.

In the laboratory the oxidation of oleic acid by the action of potassium permanganate has been carefully investigated. Saytzeff [1886] showed that dihydroxystearic acid was produced when oleic acid was treated with a dilute alkaline solution of permanganate. Lapworth and Mottram [1925], working with the same reagent, found that, if the permanganate solution were sufficiently dilute and the temperature between 0° and 10°, the conversion of oleic acid to 9 : 10-dihydroxystearic acid was almost quantitative.

By the further action of permanganate Edmed [1898] obtained nonoic, azelaic and oxalic acids, and previously Spiridonoff [1887] using the same reagent had isolated sebacic, suberic and caprylic acids. Lapworth and Mottram [1925] reconciled these results and showed that the oxidation of the dihydroxystearic acid first formed can take place in two ways according to the following equations:



The latter reaction is favoured by a more dilute solution and a lower temperature.

It is certainly improbable that an unsaturated acid containing an ethylenic linkage in the 9.10-position would in the first instance undergo oxidation in the β -position. Such migrations of the double bond as are known to take place in the fatty acids are only brought about by violent chemical reagents often acting at high temperatures. A shift from the 9.10- to the 3.4-position, for instance, is brought about when oleic acid is treated with concentrated sulphuric acid, giving rise to stearylactone, and when oleic acid is fused with caustic potash [Edmed, 1898; Le Sueur, 1901] a change of the unsaturated linkage from the 9.10- to the 2.3-position must be involved, since palmitic and acetic acids are obtained. Although this conversion of oleic to palmitic acid is exceedingly interesting, it is difficult to regard a process brought about by fusion with caustic potash as affording grounds for believing that such a change is brought about by enzyme action at the body temperature. When laboratory experiments are used as a basis for suggesting analogies with the processes actually occurring in the body, it is usual to choose reactions which can be carried out at a comparatively low temperature by the aid of mild chemical reagents such as might conceivably be present in the body. Such a method is the oxidation with hydrogen peroxide studied by Dakin and other workers, although in the case of the higher fatty acids Dakin found it necessary to carry out the reaction at 95°. Even at this temperature only about 10 % of the original acid is attacked, but Dakin [1908, 1910] succeeded in isolating the

ketones derived from the β -hydroxy-derivatives of the fatty acids by the elimination of CO_2 . He quoted these experiments as furnishing an instance of oxidation under conditions not too far removed from those obtaining in the body and advanced them in support of the view that the degradation of the saturated fatty acids takes place in the body by a repeated process of β -oxidation. However, if the β -keto-acids are formed in the body, all except β -ketobutyric acid must be immediately further broken down without the separation of the corresponding ketones, since the only fatty ketone known to occur in the body is acetone, and there is no evidence that the higher ketones would be readily attacked.

The possible conversion of the β -hydroxy-acids to $\alpha\beta$ -unsaturated acids also lacks any experimental foundation.

Clutterbuck and Raper [1925] have shown that in the action of hydrogen peroxide on the saturated fatty acids, oxidation occurs in the γ - and δ -positions at the same time as in the β -position and to about an equal extent.

Hazura [1888] investigated the oxidation of oleic acid with hydrogen peroxide in the absence of an alkali at ordinary temperature but failed to detect any evidence of oxidation.

The present communication records the results of the oxidation of this acid under the experimental conditions used by Dakin and his colleagues and also by the method described by Battie and Smedley-MacLean [1929] in which, by the addition of a cupric salt as catalyst, oxidation proceeds much more readily and at a lower temperature.

THE OXIDATION OF OLÉIC ACID WITH HYDROGEN PEROXIDE AT 95°.

4 g. of oleic acid were used in each experiment, the oxidation being carried out in slightly alkaline solution and a sufficient quantity of the peroxide added to furnish four atoms of oxygen for each molecular proportion of oleic acid. The reaction mixture was heated at 95° for 3 to 3.5 hours. Acid was then added and the CO_2 blown off and absorbed by baryta, and the separation into various fractions carried out as described on p. 1260. After the carbonic acid had been removed, the insoluble oil floating on the surface was filtered off: this formed nearly 90 % by weight of the oleic acid originally taken.

The carbonic, acetic and formic acids produced account for less than 10 % of the carbon of the oleic acid originally present. The neutral volatile distillate gave a positive reaction with sodium nitroprusside, which was probably due to acetone since we succeeded in isolating a very small amount of crystals of a dinitrophenylhydrazone which melted at 121–123°. A specimen of acetone dinitrophenylhydrazone melted at 123–124°. A trace of acetone was, therefore, probably present. A small amount of acid (0.16 g.) was obtained from the aqueous reaction mixture by continuous extraction with ether. There was too little to be purified satisfactorily, but since it gave an iodoform reaction in the cold and a molecular weight of 151 by titration, it was evidently similar to the 4-keto-7-hydroxycaprylic acid isolated in much larger quantity in subsequent

experiments when copper had been added to the reaction mixture. We were unable to detect succinic acid in the small amount of material available but we can say with certainty that not more than a trace of this acid could have been present. A trace of an acid soluble in water and insoluble in ether appeared to be oxalic acid.

The main fraction of the oil was insoluble in water. It was neutralised with $N/2$ NaOH and the aqueous solution extracted with ether to remove any neutral substances present.

The neutral fraction was present only in small amount, the total weight being about 5 % of the oleic acid originally taken. It was separated into two parts by gently warming its alcoholic solution on a water-bath and adding to it at intervals small quantities of $N/2$ NaOH until the liquid remained just alkaline. After removing the alcohol the slightly alkaline solution was extracted with ether and a small amount of a neutral substance isolated. Four specimens of the fraction, which did not dissolve on warming with dilute alkali, were obtained in different experiments and were analysed with the following results:

	% C	% H	
(4)	75.70	11.90	
(3)	75.25	11.96	
(2)	74.71	11.65	
(1)	74.25	11.79	(least pure specimen, probably contaminated by lactone)

These figures agree best with the figures required by a diketoeptadecane $C_{17}H_{32}O_2$ (C, 76.12; H, 11.90 %). Attempts to prepare an oxime or dinitrophenylhydrazone were, however, unsuccessful and the nature of this substance has not been satisfactorily settled. The only possible product of β -oxidation would, however, be contained in this fraction, so that we can say that if under the conditions of this experiment β -oxidation does take place, it can occur only to a very slight extent and is certainly not the main path of the reaction.

Acidification of the solution produced by warming the neutral fraction with dilute alkali set free an oil with a pleasant characteristic odour, which was left in a desiccator until of constant weight and then analysed. It contained C, 69.70, 69.79; H, 10.49, 10.71 %, thus agreeing with the figures required by the lactone of a ketodihydroxystearic acid, $C_{18}H_{32}O_4$, C, 69.23; H, 10.25 %. Oxidation had, therefore, probably taken place in the γ - or δ -position as well as at the ethylenic linkage.

The acid fraction was extracted by means of light petroleum from the aqueous solution after it had been acidified; a small fraction remained insoluble in light petroleum from which crystals were obtained melting at 130° ; these were identified as dihydroxystearic acid by a determination of a mixed melting-point. The composition of the oil which dissolved varied somewhat in different experiments; its iodine value varied between 48 and 58, so that if it is justifiable to regard this as due to the presence of oleic acid, from 53 to 65 % of the fraction consisted of unchanged oleic acid, *i.e.* from 40 to 50 % of the starting material had not undergone any oxidation. The presence of

oleic acid in the fraction was confirmed by oxidation with a dilute solution of potassium permanganate at 5° and the resulting dihydroxystearic acid was identified by analysis and determination of melting-point.

It was found that a separation of the fraction might be made by means of its silver salts. The oil was dissolved in alcohol, neutralised, the silver salt precipitated in boiling solution, filtered off and well washed with hot water. The insoluble silver salt was then repeatedly heated with boiling water, and the washings added to the solution of the soluble silver salt, which was then acidified and extracted with ether. The oil obtained in two separate experiments contained C, (1) 64.69, 64.65; (2) 63.01; H, (1) 9.70, 9.78; (2) 9.29 %. Mol. wt. by titration = (1) 188; (2) 198. Iodine value = 0. Calculated for ketodecoic acid, $C_{10}H_{18}O_3$, C, 64.52; H, 9.68 %. Mol. wt. = 186: for ketononoic acid C, 62.77; H, 9.42 %. Mol. wt. = 172.

A silver mirror was deposited on the sides of the tube when a solution of the sodium salt was warmed with an ammoniacal solution of silver nitrate. When heated with silver oxide and water, CO_2 was evolved and a volatile acid with a slightly goatly smell deposited on the sides of the tube. An α -ketoic acid is therefore present, a derivative of either nonoic or decoic acid. It was thought possible that the semialdehyde of azelaic acid might be present in this fraction, but no evidence was obtained of the presence of azelaic acid on oxidation.

The acid obtained from the insoluble silver salt contained from 71 to 75 % C and from 10.6 to 11.8 % H. From this fraction a very small amount of white crystals was separated melting at 49–50°, which appeared to be identical with those obtained in another series of experiments (see p. 1260), and which gave figures on analysis approximating to those required by ketostearic acid. The fraction obtained from one experiment (C, 74.03; H, 11.32 %) was converted into the zinc salt and this fractionally crystallised from boiling alcohol. The acid obtained from the least soluble portion gave figures on analysis agreeing with those for oleic acid: the composition of that from the most soluble part of the zinc salt agreed with that of a ketostearic acid (C, 72.55, 72.53; H, 10.61, 10.80 %) but, since it had an iodine value of 41, it still contained a considerable proportion of oleic acid, and we were unable to obtain from it either a satisfactory oxime or dinitrophenylhydrazone, nor did crystals separate on standing.

An attempt to calculate the composition of the oxidation product was made as follows. The acid derived from the soluble zinc salt contained 72.54 % C and 10.7 % H, and had an iodine value of 41. If the latter were due to oleic acid (C, 76.6; H, 12.05 %; i.v. 90), the following equations would give the percentage of carbon and hydrogen in the oxidation product.

$$41 \times 76.6 + 49 C = 90 \times 72.54, \text{ whence } C = 69.1 \%$$

$$41 \times 12.05 + 49 H = 90 \times 10.7, \text{ whence } H = 9.6 \%$$

These figures agree most nearly with those required for the diketostearic

acid (C, 69.23; H, 10.26 %), so that although we have not yet succeeded in isolating this substance, it is possible that it is present.

Table I. *Results of oxidation of 4.05 g. oleic acid as Na salt by 33 cc. (20 vol.) H_2O_2 in absence of a catalyst.*

Wt. CO_2 g.	Volatile acids g.	Volatile neutral. Rothera's acetone test	Fraction water-ether- soluble g.	Fraction water- soluble ether- insol. g.	Fraction water-insol. ether-sol. g.	
					Acid	Neutral
0.10	Formic 0.09 Acetic 0.48	Positive	(a) 0.11 water- sol. acid (b) 0.05 alcohol- sol. acid	0.07	<i>Petroleum-soluble:</i> (a) oleic acid 1.71 (b) diketostearic acid ? 0.81 (c) α -ketodecoic or α -keto- nonoic 0.28 <i>Petroleum-insoluble:</i> Dihydroxystearic 0.46	0.3

In his work on the oxidation of the saturated fatty acids, Dakin found that the amount of oxidation was very little affected by increasing the proportion of hydrogen peroxide. In the series of experiments about to be described in which a cupric salt was used as catalyst, a large excess of peroxide was taken and an experiment was therefore carried out in which the same excess of peroxide was used but without the addition of the catalyst. Sufficient peroxide was now present to oxidise completely the whole of the oleic acid to carbonic acid.

Carbonic, formic and acetic acids now accounted for about 18 % of the carbon of the original oleic acid: the chief product was still a colourless oil, but the greater proportion of it was now insoluble in light petroleum. The proportion of dihydroxystearic acid was much greater and more of the oleic acid had undergone oxidation.

To sum up, whereas in Dakin's investigations on the higher saturated fatty acids about 10 % of the acid was attacked, the chief products being carbonic acid and the methyl alkyl ketone formed as decomposition products of the corresponding β -hydroxy-acid, when oleic acid is similarly treated about 50 % of the acid undergoes oxidation, a considerable proportion of it being converted to a fraction corresponding in composition with diketostearic acid; α -ketodecoic acid is formed and small amounts of dihydroxystearic acid. Carbonic, formic and acetic acids account for less than 10 % of the total carbon of the original oleic acid. Less than 5 % of the oxidation product consists of a neutral fraction in which a lactone of ketodihydroxystearic acid and an oil having the empirical formula required for a diketohaptadecane were present. The latter substance was not, however, definitely identified.

THE OXIDATION OF OLEIC ACID WITH HYDROGEN PEROXIDE IN PRESENCE OF A CUPRIC SALT.

When the oxidation of oleic acid is carried out in the presence of a cupric salt, the results differ widely from those just described. Oxidation proceeds

much more rapidly and at 95°, the temperature at which Dakin carried out his experiments, the carbonic acid formed accounts for the complete oxidation of more than 50 % of the total oleic acid, another 20 % being converted to acetic and formic acids. 70 % of the original acid has, therefore, been broken down to substances containing one or two carbon atoms. The fraction of residual oil which alone contains any product of a molecular weight approaching that of oleic acid is very small. In two experiments, in each of which 4 g. of oleic acid were taken, the residual oils insoluble in water were respectively 0.03 and 0.13 g. as compared with 3.7 g. in the corresponding experiment without the catalyst. Even at 60° the amounts of carbonic, formic and acetic acids correspond to a complete conversion of 20 % of oleic acid into these substances. At this temperature, the fraction derived from the aqueous reaction mixture by continuous extraction with ether contains the largest portion of the product, *i.e.* about 2.5 g. from 4 g. oleic acid, equivalent to the conversion of 40 % of the oleic acid. This fraction consists mainly of two substances:

(a) Crystals melting at 181–182° (0.5 g.) after being once recrystallised from water.

Analysis: C, 41.53; H, 5.23 %. Mol. wt. (by Ag salt), 132.

Calculated for $C_4H_6O_4$: C, 40.68; H, 5.08 %. Mol. wt. 118.

This substance was therefore identified as succinic acid.

(b) An oil (1.7 g.) to which the formula $C_8H_{14}O_4$ was assigned. Micro-analysis of specimens obtained in two different experiments gave the following results:

C, 53.66, 53.41; H, 7.83, 7.97 %.

Mol. wt. (a) by titration, 166; (b) by iodoform estimation, 174, 189.

It was difficult to free this substance from all traces of succinic acid and the latter probably accounts for the low carbon percentage. The same substance was, however, subsequently obtained in a purer condition from the oxidation of caprylic acid (see p. 1259). A mixture of 90 % of the above acid with 10 % succinic acid would give 53.72 % C, 7.75 % H. Mol. wt. 162.

The formula $C_8H_{14}O_4$ was therefore adopted and the acid regarded as a 4-keto-7-hydroxycaprylic acid for the following reasons.

(1) It gave an iodoform reaction in the cold. This would be produced by the presence of a $—CO—$ or $—CH(OH)—$ group next to the terminal methyl group and its formation shows that oxidation must have occurred at the seventh carbon atom.

(2) The estimation of the $CH_3.CO—$ group by its conversion to iodoform corresponded with a molecular weight of 174 and 189.

(3) The amount of substance extracted by ether from the reaction mixture when the experiment had been carried out at 95° was small but consisted chiefly of succinic acid. The product when the oxidation was carried out at 60° consisted mainly of the ketohydroxycaprylic acid, the proportion of succinic acid present being small. Some of the caprylic acid derivative

appeared, therefore, to have been converted into succinic acid at the higher temperature.



(4) In the oxidation of caprylic acid itself at 60° under similar conditions the same ketohydroxycaprylic acid and succinic acid were formed, so that in this case succinic acid must have come from the eight-carbon atom chain.

(5) The acid was not readily converted to a lactone and the hydroxyl group is not therefore in the γ - or δ -position.

(6) The presence of the $-\text{CO}-$ or $-\text{CH}(\text{OH})-$ group in the α - or β -position could be excluded.

The amount of the ketohydroxycaprylic acid isolated when the oxidation was carried out at 60° corresponded to the conversion of almost exactly 70 % of the 8 terminal atoms of the oleic chain and it is improbable that the separation of this oxidation product was quantitative: it seemed most probable, therefore, that caprylic acid was formed from the oleic acid and subsequently underwent oxidation. The oxidation of caprylic acid was carried out under similar conditions and from the corresponding fraction of the product the same substance $\text{C}_8\text{H}_{14}\text{O}_4$ was isolated, together with succinic acid.

Its properties agreed with those described above.

Micro-analysis: C, 54.83; H, 7.57 %.

Calculated for $\text{C}_8\text{H}_{14}\text{O}_4$: C, 55.17; H, 8.05 %.

Mol. wt.: from titration 172; from an iodoform estimation 172. Theory 174.

Table II. *Showing results of the oxidation of 5 g. caprylic acid at 60° for 4 hours. Materials used; 32 cc. N NaOH, 110 cc. water, 15 cc. solution of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (2.73 %) and 350 cc. H_2O_2 (20 vols.).*

Product	Exp. I g.	Exp. II g.	Approximate % of total carbon calc. on mean of I-II	
<i>Carbon dioxide:</i>				
(a) during oxidation	0.50	0.33	—	
(b) during distillation	0.28	0.35	6.1	
<i>Volatile acids:</i>				
Caprylic (recovered)	0.37	0.55	9.3	Mol. wt. by titration { 147 155
Acetic, formic	1.03	0.69	10.4	Mol. wt. by titration { 79 49
<i>Soluble in water and in ether:</i>				
Succinic	3.99	3.25	59.8	Calc. as containing 55 % C
Ketohydroxycaprylic	1.80	—	—	Small amount crystals
				M.P. 178–180°. For ana-
				lysis of ketohydroxy-
				caprylic acid see above
Sparingly sol. in water, sol. in alcohol	1.03	—	—	Contains a lactone
<i>Soluble in water, insoluble in ether</i>	0.40	0.18	2.4	Mol. wt. (dibasic) 82. Gly-
				collic and glyoxylic absent.
				Chiefly oxalic
<i>Insoluble in water, soluble in ether:</i>				
(a) residual oil	0.10	0.70	6.5	Mol. wt. by titration { 178 171
(b) extracted by ether from charcoal	0.14	0.14	—	Titrate as lactone
Total			94.5 of total carbon	

When the oxidation of the oleic acid was carried out at 60° about 20 % of it was converted to a mixture of oxidised derivatives of stearic acid, varying somewhat in different experiments. These were separated into products respectively insoluble and soluble in light petroleum, and the following fractions were separated and analysed. The residual oil varied in composition in different experiments and we did not obtain sufficient completely to purify the various fractions, or to identify them satisfactorily.

Table III. *Composition of fractions of water-insoluble oil obtained by oxidation of oleic acid at 60° in presence of a cupric salt, determined by micro-analyses.*

- (1) Crystals M.P. 48–50°, soluble in light petroleum
C, 73.57; H, 11.73 %. Mol. wt. by titration 302.
Calculated for ketostearic acid: C, 72.50; H, 11.41 %. Mol. wt. 298.
M.P.: 9-ketoacid 83°; 10-ketoacid 74–76°
- (2) Crystals M.P. 84–86°, insoluble in light petroleum
C, 67.44; H, 11.25 %.
Calculated for dihydroxystearic acid: C, 68.34; H, 11.39 %.
M.P.: low-melting form 98–99°; high-melting form 130°
- (3) Oil soluble in light petroleum
C, 67.59; H, 10.41 %
- (4) Oil insoluble in light petroleum
C, 66.58; H, 9.98 %. Mol. wt.: acid from Ag salt 348; by titration 358. Oil titrates like a lactone.
Calculated for lactone of ketotrihydroxystearic acid: C, 65.87; H, 9.79 %. Mol. wt. acid 346
- (5) Oil
C, 61.48; H, 9.98 %

Method of experiment.

4 g. of pure oleic acid were neutralised with 95 % of the theoretical amount of *N* NaOH, and dissolved in 210 cc. of water to which 24 cc. of a 2.73 % solution of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ had been added. The solution was contained in a three-litre flask fitted with a reflux condenser, to the top end of which was connected (a) an inlet tube provided with a clip through which air or nitrogen could be passed into the liquid in the flask, and (b) an exit tube leading to a series of flasks containing known volumes of standard baryta solution. This was then heated on a water-bath at the required temperature. 560 cc. of 20 volume hydrogen peroxide, previously made neutral, were added, and the flask well shaken. The inlet tube was closed and the CO_2 evolved collected in the baryta flasks. After 3 to 4 hours the heating was stopped, 5 cc. of concentrated HCl or H_2SO_4 were added to the reaction mixture and a current of nitrogen passed through to drive off CO_2 , which was absorbed by the standard baryta.

The reaction mixture was filtered through a wet filter-paper which was subsequently dried and extracted with ether, any oil insoluble in water being thus separated. The filtrate was made alkaline to litmus, charcoal added and left to stand overnight so that any excess of hydrogen peroxide was destroyed. The charcoal was removed by filtration and when dry extracted with ether to remove any product which it might have absorbed. The filtrate was acidified

Table IV. Showing results of oxidation of oleic acid by H_2O_2 in presence of a cupric salt.

	CO ₂		Volatile acids		Volatile neutral. Na nitro-prusside test for acetone	Extract sol. in water and in ether g.	% of total C	Extract sol. in ether g.	% of total C	Oil insol. in water, sol. in ether g.	% of total C
	Wt. (g.)	% of total C	g.	% of total C							
At 60°											
Exp. I. 3½ hrs. 4 g. oleic acid as Na salt, 560 cc. H ₂ O ₂ (20 vol.)	During oxid. 0.67 dist. 0.30 0.97	8.6	Formic 0.10 Acetic 0.68	9.7	Positive	Total 1.56 Easily sol. in water 0.92 (a) 4-keto-7-hydroxy-caprylic acid (b) succinic acid Sol. in alcohol 0.38	—	Total 0.49 (contains approx. oxalic and glycollic)	4.6	Total 0.86	18.9 approx.
Exp. II. 3½ hrs. 4 g. oleic acid as Na salt, 560 cc. H ₂ O ₂	During oxid. 1.04 dist. 0.37 1.41	12.6	Formic 0.09 Acetic 0.65	9.4	Positive	Total 2.46 Easily sol. in water 2.15 (a) ketohydroxy-caprylic acid 1.69 (b) succinic acid 0.46 Sol. in alcohol 0.10	36.2	Total 0.76	7.2 approx.	Total 0.83	18.2
Exp. III. 3½ hrs. 4.86 g. oleic acid as NH ₄ salt, 560 cc. H ₂ O ₂	Not estimated	—	Formic 0.25 Acetic 1.11	13.7	Positive	Total 2.79 Easily sol. in water 2.59	—	Total 0.83	6.5 approx.	Total 0.63	11.4
Exp. IV. 5 hrs. 4.26 g. oleic acid as NH ₄ salt, 560 cc. H ₂ O ₂	During oxid. 0.65 dist. 0.22 0.87	7.2	Formic 0.08 Acetic 0.79	9.7	Positive	Total 2.93	—	—	—	Total 0.53	10.9
At 95°											
Exp. V. 3½ hrs. 4.1 g. oleic acid as Na salt, 560 cc. H ₂ O ₂	During oxid. 5.75 dist. 0.45 6.20	53.9	Incompletely acidified, not estimated	—	Positive faint	Total 0.40 Easily sol. in water 0.25 Sol. in alcohol 0.15	—	Total 0.27	2.5 approx.	Total 0.13	2.8
Exp. VI. 3½ hrs. 4.03 g. oleic acid as Na salt, 560 cc. H ₂ O ₂	During oxid. 5.03 dist. 0.55 5.58	50.5	Formic 0.01 Acetic 1.45	18.6	Positive	Total 0.76 Easily sol. in water 0.55 (a) ketohydroxy-caprylic acid 0.07 (b) succinic acid 0.48 Sol. in alcohol 0.09	7.6	Total 0.33	3.1 approx.	Total 0.03	0.7

and steam-distilled, the apparatus being arranged so that any CO_2 given off could be absorbed and estimated.

The distillate was neutralised and distilled to remove any neutral volatile products, the neutral distillate being tested for acetone and aldehyde. The remaining solution was evaporated to dryness and the sodium salts of the volatile acids obtained. The percentage of formate in this was estimated by the mercury method. After steam distillation, the residual non-volatile liquor was evaporated to a small bulk and subjected to continuous ether extraction for several days. The total extract obtained was evaporated to dryness and dried in a vacuum desiccator until of constant weight. The golden-brown oil remaining was extracted first with water and then with 96% alcohol, and the residues from these two extracts were separately examined.

The aqueous solution, after it had been thoroughly extracted with ether, was treated with H_2S to remove traces of copper and then evaporated to dryness and extracted with absolute alcohol. The residue contained in the alcohol was weighed and examined for glycollic, glyoxylic and oxalic acids.

DISCUSSION.

When oleic acid is treated with hydrogen peroxide in the presence of a copper catalyst, it appears that oxidation first takes place at the 9th and 10th carbon atoms and then proceeds further in the 18 carbon chain, the position of the carbon atoms next attacked not having been definitely determined; since a lactone was obtained it seems probable that oxidation had taken place in the γ - or δ -position.

The oxidised chain then breaks up, but neither azelaic nor suberic acid could be detected. Succinic and oxalic acids were the only dibasic acids isolated, only a trace of the latter being present.

The eight terminal carbon atoms of the oleic chain are broken off and the remaining part of the chain appears to be completely broken up, succinic, carbonic, formic, acetic, glycollic and oxalic acids having been identified. The amount of succinic acid formed during the oxidation must be considerably greater than that actually isolated, for it has been shown that in an acid reaction mixture succinic acid is rapidly oxidised under the conditions of the experiment [Battie and Smedley-MacLean, 1929]. During the oxidation of the oleic acid in alkaline solution probably comparatively little of the succinic acid is attacked, but after the acidification of the reaction mixture, excess of the peroxide still being present, considerable oxidation of the succinic acid will occur while the carbonic acid is being removed from the solution.

The eight terminal atoms of the oleic chain undergo oxidation, behaving apparently as caprylic acid itself behaves under similar conditions, and the 4-keto-7-hydroxycaprylic acid first formed is broken down at a higher temperature, there being evidence that succinic acid is formed as a product of this decomposition.

In the absence of the copper catalyst it is possible that a very small amount

of a ketone may have been formed such as would be expected if the ketostearic acid first formed had undergone β -oxidation, but the evidence for it was not definite, and in any case the amount was very small. The direct evidence for the β -oxidation of the higher fatty acids rests exclusively on experiments on the oxidation of stearic, palmitic and myristic acids by means of hydrogen peroxide at a temperature very little below the boiling-point of water. Experiments furnishing evidence that the animal body can directly bring about β -oxidation have all been carried out with acids containing ten or less carbon atoms. The experiments of Embden and Marx [1908], in which the liver was perfused with solutions of salts of the lower fatty acids containing up to ten carbon atoms, showed that one molecular proportion of the even-numbered fatty acids produced from 10 to 17 % of a molecule of acetone. The highest proportion (17 %) was produced from a molecular proportion of butyric and decanoic acids respectively, which is in accordance with the view that one molecule of acetoacetic acid comes from one molecule of fatty acid, being derived probably from the four terminal carbon atoms. Actually, small amounts of acetone were also derived from the odd-numbered acids, a molecular proportion of nonanoic acid furnishing 4 % of a molecule of acetone.

Again, the classical feeding experiments of Knoop were carried out with acids containing not more than six carbon atoms.

Leathes has urged that the first stage in the utilisation of the fatty acids consists of their desaturation in the liver. The results of the experiments *in vitro* now described show that when oleic acid is oxidised under these comparatively mild conditions, an eight-carbon chain fatty acid remains intact and undergoes further oxidation, but that the chain from the carboxyl group to the double bond is completely broken down into substances containing not more than two carbon atoms with the possible exception of succinic acid. The actual isolation from oleic acid of caprylic acid derivatives disposes of the objection raised by Leathes and Raper [1925, p. 194] that oleic acid would first be broken down into pelargonic and azelaic acids, and that as these contain odd numbers of carbon atoms they would not be expected to furnish acetoacetic acid.

The evidence now presented from laboratory experiments has been obtained under conditions probably more nearly approaching those existing in the body than those used in previous work on the oxidation of oleic acid. The action of a mild oxidising agent such as hydrogen peroxide at 60° is not very far removed from conditions *in vivo* and even copper is known to occur in marked amount in liver tissue [Morrison and Nash, 1930].

The fact that the introduction of a cupric salt as catalyst makes such a marked difference in the course of the oxidation of fatty acids by hydrogen peroxide, in the case of caprylic acid replacing the β -oxidation described by Dakin by γ -oxidation and in the case of caprylic and oleic acids producing a considerable proportion of succinic acid, is of some interest when its biological application is considered.

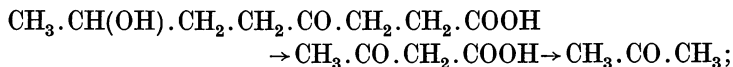
The wide distribution in the body of the powerful specific enzyme succinoxidase [Batelli and Stern, 1911; Thunberg, 1909] has so far found no sufficient explanation, for, as Clutterbuck [1927] has pointed out, its only function appears to be to deal with the comparatively small amount of succinic acid derived from glutamic acid.

The conversion of 50 % of butyric into succinic acid [Cahen and Hurtle, 1917], the production of γ - and δ -keto-acids from the higher saturated fatty acids [Clutterbuck and Raper, 1925] and the conversion of the δ -keto-acids to succinic acid led Clutterbuck to suggest succinic acid as a possible intermediary between fat and carbohydrate.

The experiments now described show that under mild conditions of oxidation succinic acid may be formed in considerable quantity from oleic acid, and in the body this product would be further oxidised by the succinoxidase of the liver and muscles to malic acid.

A considerable body of evidence is accumulating that there is a widespread mechanism for dealing with the oxidation of succinic acid, not only in the animal body but also in lower organisms. Stent, Subramaniam and Walker [1929], for instance, have shown that *Aspergillus niger* contains enzymes by which succinic is converted through malic to oxaloacetic acid and point out that the conversion of the latter to pyruvic and lactic acids would form a bridge over which one might pass from fat to carbohydrate. They also found that when *A. niger* acts on butyric acid small amounts of succinic acid are formed; they appear to prefer the view that these are formed rather by dehydrogenation of acetic acid derived from the oxidation of the butyric acid than by direct oxidation of the butyric acid. We carried through an oxidation of acetic acid under conditions similar to those we used for the oxidation of oleic acid but were unable to extract any succinic acid from the product: therefore the succinic acid produced in our experiments is certainly not derived from acetic acid.

In all our experiments on the oxidation of oleic acid traces of acetone have been identified in the neutral volatile product by means of the sodium nitroprusside reaction, and in one case this has been definitely confirmed by the preparation of a crystalline derivative. It is possible that the source of the acetone might be the 4-keto-7-hydroxycaprylic acid, which might suffer the following degradation:



or the caprylic acid residue might undergo oxidation, leaving a butyric acid residue which might then be oxidised further, or the acetone might be derived from the remaining part of the oleic molecule. In any case the amount formed is only just detectable and possibly represents the product of some alternative path of oxidation.

The evidence that β -oxidation of the lower fatty acids can take place in

the body is convincing, but it does not necessarily follow that this is the only method of oxidation of the saturated fatty acids nor that it is the method by which the unsaturated acids undergo oxidation. The classical feeding experiments of Knoop which have led to the general acceptance of the β -oxidation theory have the disadvantage that they had to be carried out on the phenyl derivatives of the acids. In this case it would be impossible for the terminal butyric residue to be converted to succinic acid, since it would be a benzyl and not a methyl group that would be available for oxidation. Obviously, considerable caution must be exercised in accepting conclusions based on acids in which the terminal methyl is replaced by a benzyl group.

It is possible that alternative paths of oxidation exist in the body, so that the acids may be broken down either by a process of β -oxidation leading to the production of acetoacetic acid, or by a process involving γ -oxidation such as is now shown to occur in the laboratory with caprylic acid leading to the formation of succinic acid.

It may be that a catalyst exists in the body playing the part of the cupric salt *in vitro*, and that it is the failure of this catalyst to function effectively in starvation and in diabetes which leads to the production of large quantities of the acetone bodies.

Perhaps the solution of that mysterious problem, the manner in which "fat burns in the fire of carbohydrate" is to be sought in the effect of a liver stored with glycogen upon the oxidative mechanism. It is not impossible that the determining factor as to whether β - or γ -oxidation may occur is to be looked for in the nature of the liver cells and that the particular condition of the cell may be connected with its glycogen content.

In any case the possible degradation of oleic acid through succinic rather than through β -hydroxybutyric acid finds some support from the results now brought forward.

We are now investigating the influence of the copper catalyst on the oxidation of the higher saturated fatty acids by means of hydrogen peroxide.

SUMMARY.

1. When the oxidation of oleic acid was carried out by hydrogen peroxide at 95° under the conditions used by Dakin in his experiments on the oxidation of the higher saturated fatty acids, a fraction, agreeing in composition with that of diketostearic acid, was formed, and evidence of the presence of an α -keto-derivative of nonoic or decoic acid was obtained. Some dihydroxystearic acid was also present.

2. The carbonic, acetic and formic acids produced corresponded to the complete oxidation of from 5 to 10 % of the total oleic acid. Increasing the proportion of hydrogen peroxide increased the proportion of dihydroxystearic acid present.

3. The addition of a small amount of cupric salt greatly increased the extent of the oxidation. At 95° with a large excess of hydrogen peroxide, more

than 70 % of the original oleic acid was completely broken down to carbonic, formic and acetic acids. Succinic and 4-keto-7-hydroxycaprylic acids were also identified.

4. When the oxidation in the presence of a cupric salt was carried out at 60°, the amounts of carbonic, formic and acetic acids produced corresponded to the complete oxidation of about 20 % of the total oleic acid. The product obtained in greatest amount was a derivative of caprylic acid to which the formula 4-keto-7-hydroxycaprylic acid has been assigned. A considerable proportion of succinic acid was also present. A small quantity of an oil, insoluble in water, consisted of a mixture of various oxygenated derivatives of stearic acid, the composition of which varied in different experiments.

5. Among the products of oxidation of caprylic acid were succinic acid and the same 4-keto-7-hydroxycaprylic acid as was previously obtained from oleic acid.

6. The bearing of these results on the theory of β -oxidation is discussed.

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